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New methods for capturing the mystery lipid, PtdIns5P

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Abstract

The enormous versatility of phosphatidylinositol as a mediator of intracellular signalling is due to its variable phosphorylation on every combination of the 3', 4' and 5' positions, as well as an even more complex range of phosphorylated products when inositol phosphate is released by phospholipase C activity. The phosphoinositides are produced by distinct enzymes in distinct intracellular membranes, and recruit and regulate downstream signalling proteins containing binding domains [PH (pleckstrin homology), PX (Phox homology), FYVE etc.] that are relatively specific for these lipids. Specific recruitment of downstream proteins presumably involves a coincidence detection mechanism, in which a combination of lipid–protein and protein–protein interactions define specificity. Of the seven intracellular phosphoinositide, quantification of PtdIns5P levels in intact cells has remained difficult. In this issue of the *Biochemical Journal*, Sarkes and Rameh describe a novel HPLC-based approach which makes possible an analysis of the subcellular distribution of PtdIns5P and other phosphoinositides.

DISCOVERY AND ANALYSIS OF PtdIns5P

PtdIns5P was first identified in 1997 by Rameh, Cantley and colleagues [1]; studies on the lipid have been reviewed previously [2]. It is not yet known whether a specific phosphatidylinositol 5-kinase exists; the ability of the PtdIns3P 5-kinase PIKfyve to produce PtdIns5P *in vivo* has been controversial [3,4]. Alternatively, PtdIns5P can be produced by dephosphorylation of the 4' position of PtdIns(4,5)P₂ by the type I and II PtdIns(4,5)P₂ 4-phosphatases [5], or by removal of the 3' phosphate from PtdIns(3,5)P₂ by one of the myotubularin family of lipid phosphatases [6]. It can be degraded by a PTEN (phosphatase and tensin homologue deleted on chromosome 10)-related phosphatase, PLIP (PTEN-like phosphoinositide 5 phosphatase), which is specific for PtdIns5P [7,8].

While the intracellular accumulation of phosphoinositides is often measured using fluorescently labelled lipid-binding domains, these methods have serious limitations owing to the poorly defined role of protein–protein interactions and lipid headgroup availability in the targeting of these probes [9]. The *sine qua non* method for quantification of phosphoinositides involves extraction and deacylation of lipids from metabolically labelled cells, followed by separation using ion-exchange HPLC. This method has been very successful for analysis of cellular production of PtdIns(4,5)P₂, PtdIns4P and the 3-

phosphoinositides [PtdIns(3,4,5) P_3 , PtdIns(3,4) P_2 and PtdIns3 P]. However, the available methods do not adequately separate PtdIns5 P from the much more abundant PtdIns4 P , greatly hampering the analysis of this lipid. Thus the most commonly used method is an indirect mass assay, in which endogenous PtdIns5 P is converted by recombinant PtdIns5 P 4-kinase into PtdIns(4,5) P_2 , which can be quantified by thin-layer chromatography [10].

The roadblock in the quantitative study of cellular PtdIns5 P levels has been overcome in the present paper in the *Biochemical Journal* by Sarkes and Rameh [10a], which describes a modification of the standard procedure that greatly enhances the separation of the deacylation products of the three monophosphoinositides (PtdIns3 P , PtdIns4 P and PtdIns5 P). Using a novel optimization of column bed volume and elution buffer, this new methodology achieves essentially baseline separation of PtdIns5 P from PtdIns4 P . The new method allows unambiguous quantification of intracellular PtdIns5 P for the first time.

CELLULAR FUNCTIONS OF PtdIns5 P

The cellular functions of PtdIns5 P are not well understood. PtdIns5 P has been implicated in p53-mediated responses to DNA damage by the identification of a nuclear tumour suppressor, ING2, as a PtdIns5 P -binding protein [11]. PtdIns5 P binding is mediated by a zinc finger domain, the PHD domain, which is also found in acetyltransferases and chromatin remodelling proteins. The ability of ING2 to modulate p53 acetylation and apoptotic responses to cell-death stimuli requires an intact PHD domain, thereby implicating PtdIns5 P in these responses. In fact, nuclear PtdIns5 P levels are regulated, as they increase substantially during G₁-phase in synchronized erythroleukaemia cells [12].

A number of studies have suggested roles for PtdIns5 P in the regulation of vesicular trafficking. Laporte and colleagues [13], using a comparative genomics approach, identified genetically linked groups comprising a lipid kinase and lipid phosphatase, many of which would lead to interconversion of PtdIns(4,5) P_2 and PtdIns(3,5) P_2 with PtdIns5 P . The analysis suggests a probable role of PtdIns5 P in membrane trafficking. Consistent with this hypothesis, insulin-stimulated translocation of GLUT4 (glucose transporter 4) is disrupted by overexpression of the PtdIns5 P -binding ING2 PHD domain, and stimulated by microinjection of PtdIns5 P [14]. PtdIns5 P also appears to play a role in the regulation of EGF (epidermal growth factor) receptor trafficking [2]. If PIKfyve can in fact produce PtdIns5 P *in vivo*, then the role of PIKfyve in endocytic regulation could in part be due to signalling by PtdIns5 P [4].

Finally, PtdIns5 P has been implicated in bacterial invasion by *Shigella flexneri*, *Salmonella dublin* and *Salmonella typhimurium*. These organisms express virulence factors that have PtdIns(4,5) P_2 4-phosphatase activity, hydrolysing PtdIns(4,5) P_2 to produce PtdIns5 P [15]. While the data suggest a positive role for PtdIns5 P in bacterial invasion, the hydrolysis of PtdIns(4,5) P_2 by these phosphatases, and subsequent disruption of the actin cytoskeleton, also plays a major role [16].

Intracellular distribution of PtdIns5P: an important step in defining its function

Using their new chromatography method, Sarkes and Rameh [10a] have produced the first direct biochemical analysis of the subcellular distribution of PtdIns5P, showing significant levels in the both Golgi and plasma membrane fractions. The function of PtdIns5P in the plasma membrane is not clear at this time, but it could involve novel functions. Now that its production there can be measured, new studies on the modulation of plasma membrane PtdIns5P in response to changes in growth-factor stimulation, stress, nutrient availability and other factors should be forthcoming. The finding of PtdIns5P in the Golgi is consistent with the established role of PtdIns4P/PtdIns(4,5)P₂ metabolism in this organelle, and may suggest a role for PtdIns5P in secretory trafficking. It is also consistent with the presence of the PtdIns5P phosphatase PLIP in the Golgi in *Dictyostelium* [7].

A side result of the analysis of monophosphoinositides in HeLa cells is the finding of a marked enrichment of PtdIns3P in the Golgi, almost twice the amount found in fractions containing the endosomal marker Rab5. This finding is in conflict with studies using FYVE domains to localize PtdIns3P in live and fixed cells [17]. The source of Golgi PtdIns3P could be either Class II or Class III PI3K (phosphoinositide 3-kinase); the latter have been observed in the Golgi, but this has been controversial [18,19]. With the new methodology described by Sarkes and Rameh [10a], which also enhances separation of PtdIns3P from PtdIns4P, analysing the effect of Class II or Class III PI3K-knockdown on Golgi PtdIns3P levels in cells is now a feasible and important experiment.

Abbreviations used

PI3K	phosphoinositide 3-kinase
PLIP	PTEN-like phosphoinositide 5 phosphatase
PTEN	phosphatase and tensin homologue deleted on chromosome 10

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